originally filed. Lastly, Applicants have added new Claims 16-22. Support for new Claims 16-22 can be found in Claims 1-11, as originally filed.

No new matter has been added. Claims 3-22 are pending in this application.

REMARKS

Present Claims 3-9 relate to methods for isotopically labeling a functional group possessed by an amino acid residue of a protein, comprising the step of reacting a transglutaminase with said protein in the presence of an isotope-labeled ammonium salt.

Present Claims 10 and 11 relate to proteins containing an amino acid residue with a functional group isotopically labeled by such a method.

Present Claims 16-22 relate to isotopically labeled proteins, prepared by a process, comprising reacting a transglutaminase with a protein in the presence of an isotope-labeled ammonium salt.

The inventors have discovered that the presently claimed methods are particularly effective for isotopically labeling a functional group in a protein. The cited references contain no disclosure or suggestion of the presently claimed methods. Accordingly, these references cannot affect the patentability of the present claims.

The rejection of Claims 1, 2, and 10 under 35 U.S.C. § 102(b) in view of Iwanij (1977); the rejection of Claims 1, 2, and 10 under 35 U.S.C. § 102(b) in view of U.S. Patent No. 4,582,794 (Russell); the rejection of Claims 1 and 10 under 35 U.S.C. § 102(b) in view of Wilbur 1992; the rejection of Claims 1, 2, and 10 under 35 U.S.C. § 102(b) in view of U.S. Patent No. 5,846,998 (Schieven); and the rejection of Claims 1, 2, and 10 under 35 U.S.C. § 102(e) under 35 U.S.C. § 102(e) view of U.S. Patent No. 6,146,842 (Josiah) have all been obviated by appropriate amendment. As the Examiner will note, Applicants have canceled

Claims 1 and 2 and amended Claim 10 such that it now depends from Claim 3. Accordingly, these rejections are no longer proper and should be withdrawn.

The rejection of Claims 1-11 under 35 U.S.C. § 103(a) in view of Iwanij (1977) and Russell and U.S. Patent No. 5,658,605 (Soeda et al) is respectfully traversed. Iwanji (1977) discloses the use of transglutaminase (herein after referred to as "TG") to label proteins using ^{3}H-putrecine or ^{14}C-glycine ethyl ester.

Soeda et al discloses that TG is may be used for introducing primary amines, for example, ammonia. However, this reference does not disclose that TG can be used for labeling a protein in the presence of ammonium salts only if the concentration of ammonium salts is low. Moreover, this reference does not disclose by what mechanism and to which degree ammonia can be introduced and does not disclose the application to functional analysis and the structural analysis of the isotopically labeled protein.

Russell discloses the use of TG to incorporate ^{3}H or ^{14}C labeled polyamines.

In contrast, the amended claims do not use labeled putrecine or labeled glycine ethyl ester as an amine donor. Instead, the present claims recite the use of labeled ammonium salts. Thus, the present inventions differ from those described in the reference.

In this regard, it must be noted that the methods disclosed in Iwanji (1977) and Russell alter the structure of the target protein, which makes it impossible to use the labeled protein for structural analysis of the native protein. In contrast to these techniques, the presently claimed methods represent a significant improvement in that they do not alter the structure of the target protein, which makes the analysis of the native proteins possible, because the amine groups appearing in the target proteins will be simply replaced with labeled ammonia.

Furthermore, the precise mechanism of ammonia incorporation has not been elucidated prior to the present invention, as discussed in more detail below. In fact, even

those skilled in the art might consider that ammonia has an inhibitory effect on TG activity, as the Examiner recognized on page 3 of the Official Action. Thus, it would not have been obvious to one skilled in the art that ammonia could be effectively incorporated into a target protein by TG. Additionally, with the present invention it is possible to easily label a protein on the μg scale, without altering its structure, and to use the labeled protein for structural analysis such as NMR analysis, even in the case where it is impossible to label a protein by expressing the protein, for example, in the case of analyzing unknown samples.

For all of these reasons, the rejection is improper and should be withdrawn.

The rejection of Claims 3-9 under 35 U.S.C. § 112, first paragraph, is respectfully traversed. On page 3 of the Official Action, the Examiner cites Takagi et al (1986) in support of the assertion that ammonium is an inhibitor of TG. In Takagi et al (1986), TG changed its substrate from dansyl cadaverine to ammonium sulfate in dose-dependent manner when the concentration of ammonium sulfate increased. However, since Takagi et al focused their study on "dansyl cadaverine incorporation", they did not notice the behavior or activity of TG on ammonium sulfate. It may be true that the incorporation of dansyl cadaverine into acetylated casein was inhibited by ammonium sulfate, but this does not mean that ammonium sulfate is an inhibitor of TG activity.

Since TG can use both dansyl cadaverine and ammonium salts as a substrate, the apparent dose-dependent inhibitory effect on transglutaminase, reported in Takagi et al (1986), should be due to the competitive reaction. Namely, the inhibitory effect observed in Takagi et al (1986) was an artifact due to their failure to determine the incorporation of added ammonium sulfate. Indeed, Takagi et al reported the inhibitory effects of ammonia on the reaction, but Takagi et al (1986) does not demonstrate any inhibitory effects of ammonium salts on TG activity and the use of TG for labeling protein in the presence of ammonium salts.

Thus, Takagi et al (1986) does not provide any information about the mechanism, possibility or difficulty of labeling a protein in the presence of ammonia or ammonium salts.

As noted above, Soeda et al disclose that TG is known as an amine introducing system for introducing primary amines, for example, ammonia. However, this reference does not disclose that TG can be used for labeling a protein in the presence of ammonium salts only if the concentration of ammonium salts is low. Furthermore, this reference does not disclose by what mechanism and to which degree ammonia can be introduced and does not disclose the application to functional analysis and the structural analysis of the isotopically labeled protein. Signorini et al (1991) is similar. Thus, these references do not provide any information about the mechanism and the possibility or difficulty of using TG for labeling a protein in the presence of ammonia or ammonium salts.

As is apparent from the above discussion, these references do not establish an undue burden of experimentation would be required for determining whether TG from other sources could be used for the presently claimed methods.

On the other hand, in the present invention, the degree of labeling, namely the degree of ammonia incorporation was examined in detail. As a consequence, the inventors found that label could be efficiently introduced into the target proteins, which labeled proteins could be used for functional or structural analysis (see for example, page 4, lines 13-15 and page 13, line 5-24). The inventors also submit that the ability of labeling a protein in the presence of ammonium salts or ammonia is a general feature of TGs. To demonstrate that those skilled in the art may use any transglutaminase for the present application, Applicants submit Nobuhisa Shimba et al., J. Agric Food Chem., vol. 50, pp. 1330-1334 (2002), a copy of which is attached hereto as Exhibit A, in which it is described that FTG (fish-derived transglutaminase) and GTG (guinea pig liver-derived transglutaminase) also catalyze the reaction in the presence of high NH_4Cl (see MATERIAL AND METHODS, as well as

RESULTS AND DISCUSSION). In this connection, it should also be noted that FTG and GTG are calcium-dependent, while MTG (microbial transglutaminase) is calcium-independent (Shimba et al, (2002), page 1330, left column, lines 15-23). As can be seen in the paper, different TGs are shown to be useful for the presently claimed methods. Accordingly, no undue burden of experimentation is required to carry out the full scope of the presently claimed methods.

Thus, the rejection is improper and should be withdrawn.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Stephen G. Baxter, Ph.D.
Registration No. 32,884

22850

Customer Number 22850
Tel. (703) 413-3000
Fax. (703) 413-2220
(OSMMN 10/01)

SGB/rac

MARKED-UP COPY OF AMENDMENT FILED HEREWITH

IN THE CLAIMS

Please cancel Claims 1 and 2, without prejudice toward the further prosecution of these claims in a continuation and/or divisional application.

Please amend the claims as shown on the attached marked-up copy to read as follows:

--3. (Amended) A method for isotopically labeling a functional group possessed by an amino acid residue of a protein, comprising the step of reacting [The method of claim 1, wherein the enzyme is] a transglutaminase[, the amino acid residue is a glutamine residue and the isotope-labeling compound is an] with said protein in the presence of an isotope-labeled ammonium salt.

4. (Amended) The method of [claim 1] Claim 3, wherein [the enzyme is a transglutaminase, the] said amino acid residue is a glutamine residue[,] and [the] said functional group is a γ-carboxamido group [and the isotope-labeling compound is an ammonium salt].

5. (Amended) The method of [claim] Claim 3, wherein [the] said transglutaminase is calcium-independent.

6. (Amended) The method of [claim] Claim 3, wherein [the] said transglutaminase is calcium-dependent and [the reaction of the] said reacting said transglutaminase with [the] said protein is conducted in the presence of calcium.

7. (Amended) The method of [claim] Claim 3, wherein [the] said transglutaminase is reacted with [a] said protein in an [aquatic] aqueous environment at [the] a pH of about pH5.0

to pH9.0 and [the] a temperature of 4°C to 55°C for a time of about 30 seconds to about 2 days.

8. (Amended) The method of [claim] Claim 3, wherein the ratio of the concentration of [the] said ammonium salt to the concentration of [the] said protein to be labeled is more than about 10.

9. (Amended) The method of [claim] Claim 8, wherein the concentration of [the] said protein to be labeled is about 1μM to about 40mM and the concentration of [the] said ammonium salt is about 10μM to about 10M.

10. (Amended) A protein [whose] containing an amino acid residue [has] with a functional group isotopically labeled according to the method of [claim 1] Claim 3.

11. (Amended) A protein [whose] containing a glutamine residue [has] with a functional group isotopically labeled according to the method of [claim] Claim 3.--

Please add the following new claims:

--16. (New) to 22. (New)--